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No Prognostic Significance of p53 Expression in Esophageal Squamous Cell Carcinoma

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Background and Objectives: It is generally accepted that the overexpression of p53 protein is associated with poor prognosis in breast, colorectal, and other types of cancer. However, the prognostic significance of p53 aberrations in esophageal squamous cell carcinoma has yet to be determined. We attempted to analyze the relationship between p53 expression and the clinicopathologic features of esophageal squamous cell carcinoma by reviewing the medical records of a large patient population. Our study of esophageal squamous cell carcinoma involves the largest patient population to date.

Methods: p53 expression in formalin-fixed, paraffin-embedded samples of 239 patients with primary esophageal squamous cell carcinoma (TNM stage I:79 cases, stage II: 82 cases, stage III: 78 cases), who underwent esophageal resection without additional treatment, were analyzed by immunohistochemical staining using a polyclonal antibody, RSP53. The relationships between p53 immunoreactivity and prognostic factors were determined by the χ^2 test, and the prognostic impact of p53 protein expression was analyzed by univariate and multivariate survival analyses.

Results: In 115 (48.1%) of 239 esophageal tumors, nuclear immunoreactivity for the p53 protein was detected. The expression of the p53 protein did not correlate with sex, age, histological grading, lymph node metastasis, vascular invasion, or TNM stage. Similiarly, p53 expression did not correlate with prognosis in univariate and multivarite survival analysis.

Conclusions: The expression of the p53 gene product had no impact on the prognosis of esophageal squamous cell carcinoma.

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KEY WORDS: **p53; prognosis; squamous cell carcinoma; esophagus**

INTRODUCTION

The p53 gene encompasses 16–20 kb of cellular DNA located on the short arm of human chromosome 17 [1,2]. This gene encodes a 393–amino acid nuclear phosphoprotein involved in the regulation of cell proliferation and acts as a tumor suppressor gene. Recent research has also revealed that wild-type p53 regulates the transition from the G1 to the S phase of the cell cycle and plays a role in determining apoptotic cell death [3,4].

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Alterations of the p53 gene or its protein have been detected in diverse human malignancies by immunohistochemistry (IHC) [5–7]. The expression of the p53 gene was associated with poor prognosis in breast [8], colon [9], stomach [10], and lung [11] cancers. However, the prognostic significance of p53 aberrations in esophageal squamous cell carcinoma (ESCC) has yet to be determined. Shimaya et al. [12] reported on a series of 105 patients in which p53 accumulation was a significant prognostic indicator in ESCC. However, Sarbia et al. [13] reported on a series of 204 patients in which p53 accumulation was not associated with a shorter survival.

In the present study, we investigated p53 protein mutation by IHC in 239 cases of ESCC. The purpose of this investigation was twofold: to evaluate correlation between overexpression of p53 and the clinicopathologic features of disease and to assess the prognostic significance of p53 expression.

MATERIALS AND METHODS

Subjects

The present study involved 239 patients with ESCC (TNM stage I:79 cases, stage II:82 cases, stage III:78 cases). All patients underwent curative resection without additional treatment at the National Cancer Center Hospital, Tokyo, between 1987 and 1996. The patients were 216 men and 23 women, ages 40–86 years (mean, 62.4). Clinical data, including histological grade, were available for all patients. The pathological stage of esophageal carcinoma was defined according to the TNM system [14]. Each lesion was graded histologically according to the World Health Organization classification [15]. The average follow-up time was 42 months (range, 2–116).

Immunohistochemical Staining of p53

An antibody directed at the p53 protein, RSP53 (Nichirei, Tokyo, Japan), was used for IHC. RSP53 is a rabbit polyclonal antibody recognizing a linear epitope in human p53, located between amino acids 54 and 69. RSP53 successfully detected p53 protein in immunoblot analysis and recognized both wild-type and mutant-type p53 proteins. Sections (5 μl) of formalin-fixed, paraffin-embedded tissue were deparaffined in xylene and rehydrated through a graded alcohol series. The sections were then incubated in 0.3% H_2O_2 in methanol for 20 min to extinguish any endogenous peroxidase activity, rinsed with phosphate-buffered saline (PBS), and incubated in a microwave oven at 90°C for 20 min. In order to reduce nonspecific background staining, 10% normal swine serum (blocking buffer) was placed on the sections for 10 min at room temperature, then drained off. The sections were then incubated with RSP53, diluted to 1:2,000 with blocking buffer, and allowed to sit overnight at room temperature. The sections were rinsed with PBS and incubated with avidin-biotin peroxidase complex for 30 min at room temperature. Finally, the sections were rinsed with PBS and developed by immersion in 0.06% 3.3′-diaminobenzidine tetrahydrochloride for 10 min, counterstained with Meyer-hematoxylin, and dehydrated by being passed through a graded alcohol series and xylene.

Sections of a breast cancer known to contain mutant p53 protein were used as a positive control. A section of the same breast tumor incubated in PBS instead of the primary antibody was included as the negative control. Each slide was examined under a light microscope without knowledge of the patient's data. The approximate percentages of p53-positive nuclear staining within the carcinomas were graded as follows: negative (−), <10%, 10–50%, or >50%.

Statistical Analysis

Frequency distributions of all investigated prognostic factors were analyzed by the χ^2 test. The survival curves for the 207 patients were expressed as described by Kaplan-Meier methods [16], with statistical significance determined by the generalized Wilcoxon test. Thirty-two patients who died of operative complications (14) or other diseases (18) were excluded. The prognostic significance of individual parameters in multiparametric analyses was determined by means of a Cox's proportional hazards regression analysis [17]. $P <$ than 0.05 was considered significant.

RESULTS

The RSP53 polyclonal antibody detected p53 protein expression in 115 (48.1%) of the 239 esophageal squamous cell carcinomas. All immunostaining for p53 protein was confined to the tumor cell nuclei (Fig. 1). Ninety (78.2%) of the 115 carcinomas demonstrated high level p53 expression, with >50% of malignant cells showing positive staining. Twenty-one of 115 tumors (18.3%) showed positive staining of 10–50% of malignant cells, and 4 tumors (3.6%) showed positive staining of <10% of malignant cells.

Correlations of p53 expression with the clinicopathologic parameters of ESCC are shown in Table I. p53 expression did not correlate with patient age, sex, histological grading, lymph node metastasis, vascular invasion, or TNM stage.

In univariate analysis, no correlation between p53 immunoreactivity and postoperative survival time was observed in patients with ESCC (Fig. 2). Similiarly, the survival curve revealed no significant difference in the mutation positive group and the negative group between different TNM stages.

In multivariate analysis according to the Cox proportional hazards model, positive lymph node metastasis ($P < 0.01$) and vascular invasion ($P < 0.01$) were significantly correlated with overall survival. None of the other parameters, including p53 immunoreactivity, had a significant effect on prognosis (Table II).

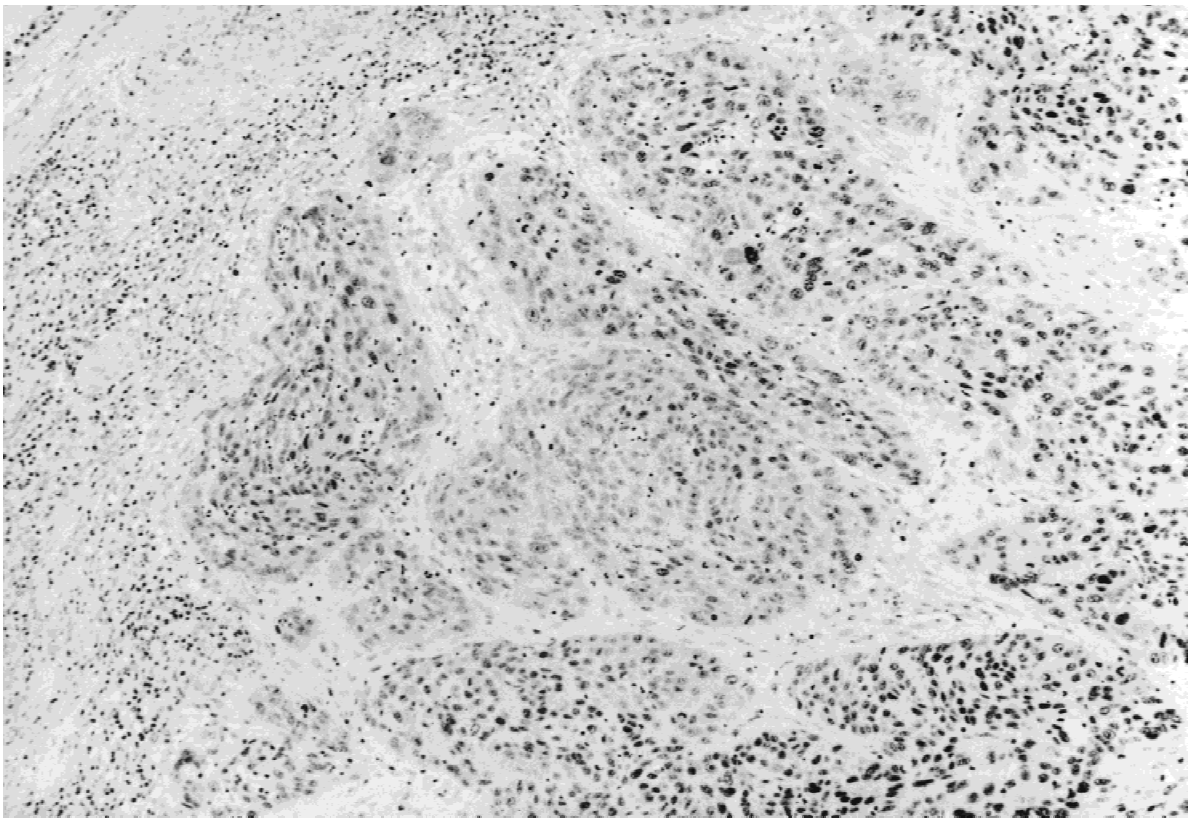

Fig. 1. Immunohistochemical staining of p53 protein using the polyclonal RSP53 antibody. Intense nuclear staining can be seen in the majority of the tumor cells of a esophageal squamous cell carcinoma.

TABLE I. Relationship Between Clinicopathological Factors and p53 Expression

Factors	p53 expression, no. (%) of cases: Positive [n = 115] (%)	Negative [n = 124] (%)	P
Sex, M/F	104/11	112/12	0.97
Patient age			0.66
<50 years	10 (8.7)	13 (10.4)	
50–60 years	57 (49.6)	66 (53.3)	
>60 years	48 (41.7)	45 (36.3)	
Histological grade			0.22
Well differentiated	43 (37.4)	34 (27.4)	
Moderately differentiated	54 (47.0)	64 (51.6)	
Poorly differentiated	18 (15.6)	26 (21.0)	
Lymph node metastasis, +	61 (53.0)	70 (56.4)	0.59
Vascular invasion, +	35 (30.4)	41 (33.0)	0.66
Lymphatic invasion, +	69 (60.0)	86 (69.3)	0.13
TNM stage			0.36
Stage I	38 (33.0)	41 (33.1)	
Stage II	35 (30.4)	47 (37.9)	
Stage III	42 (36.6)	36 (29.0)	

DISCUSSION

In the current study, p53 immunoreactivity was found in 115 (48.1%) of 239 cases of ESCC. These result are consistent with previous studies on ESCC using IHC techniques to demonstrate p53 accumulation, in which p53 immunoreactivity was detected in 34–67% of cases [12,13,21,22].

No significant correlations were found between nuclear p53 staining in ESCC and the TNM stage in the present study. Frequent expression of p53 was already evident in ESCC at stage I, and the incidence of cases positive for nuclear p53 staining did not increase with advancing stages. These findings indicate that p53 gene abnormalities are a relatively early event in esophageal carcinogenesis and are not related to tumor progression and metastatic spread. Sarbia et al. [13] recently reported that p53 immunoreactivity did not correlate with pT category, pN category, or pM category in a 204-case study of ESCC. In addition, concurrent expression of p53 protein in esophageal precancerous and preinvasive lesions has also been described [18, 19]. Moreover, elevated levels of p53 protein have been found in histologically normal mucosae of esophageal biopsies from asymptomatic individuals at high risk for esophageal carcinoma development [18, 20]. These data indicate that p53 alterations may precede the morphological changes seen during tumor development.

The prognostic significance of p53 expression by IHC using monoclonal antibody in ESCC has produced conflicting results. Three groups [12,21,22] proposed p53

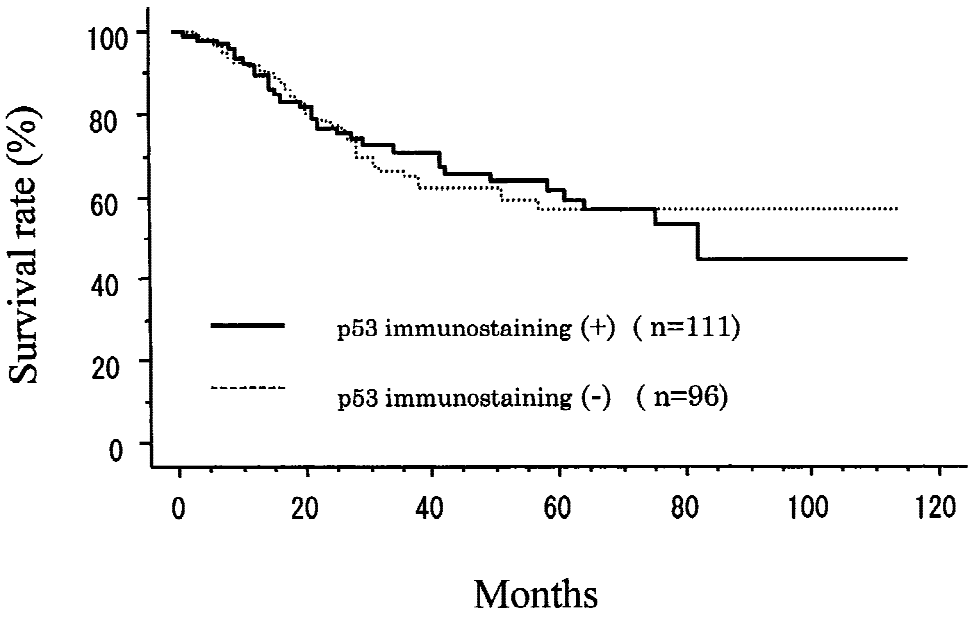

Fig. 2. Kaplan-Meier cumulative survival curves for 207 patients according to p53 expression.

TABLE II. Multivariate Results in Cos Proportional Hazards Analysis*

Variables	HR	95% CI	*P*
Positive lymph node	2.60	1.37–4.91	<0.01
Vascular invasion	2.14	1.32–3.46	<0.01
p53 expression	1.02	0.64–1.61	0.94

*HR = hazard ratio, CI = confidence interval. Differentiation grade, lymphatic invasion, age, and sex were also examined, but $P < 0.05$ for these variables.

expression as a significant prognostic indicator in ESCC. Multivariate analysis to confirm the independent prognostic value of p53 expression was not performed. In contrast, two groups [13,23] reported that p53 expression did not correlate with prognosis, either in univariate or multivariate analysis of survival.

The discrepancies between these results may be due to differences in laboratory techniques (e.g., the use of different monoclonal antibodies to screen for p53 expression), or a bias in patient populations. In our present study, an equal number of patients with TNM stages I, II, and III disease were selected as the population. All patients were treated surgically without adjuvant or neoadjuvant therapy. p53 expression was then screened for by use of a polyclonal antibody (RSP53).

The current study supports the conclusion that p53 expression is not associated with lower patient survival. Univariate analysis and multivariate analysis according to the Cox proportional hazards model did not demonstrate an independent prognostic value for p53 expression. Therefore, p53 expression cannot serve as a prognostic indicator to guide patient management in ESCC, at least for patients who undergo surgery without adjuvant therapy.

CONCLUSIONS

It seems that p53 gene abnormalities are a relatively early event in esophageal carcinogenesis and are not related to tumor progression and metastatic spread. The expression of the p53 gene product has no impact on the prognosis of esophageal squamous cell carcinoma.

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